

Evolution and Development: Rise of the Little Squirts

Dispatch

Anthony Graham

It used to be thought that only vertebrates possess neural crest cells, but a recent study has demonstrated the existence of neural crest-like cells in an ascidian urochordate. This alters our views on the evolution of the neural crest and of the vertebrates.

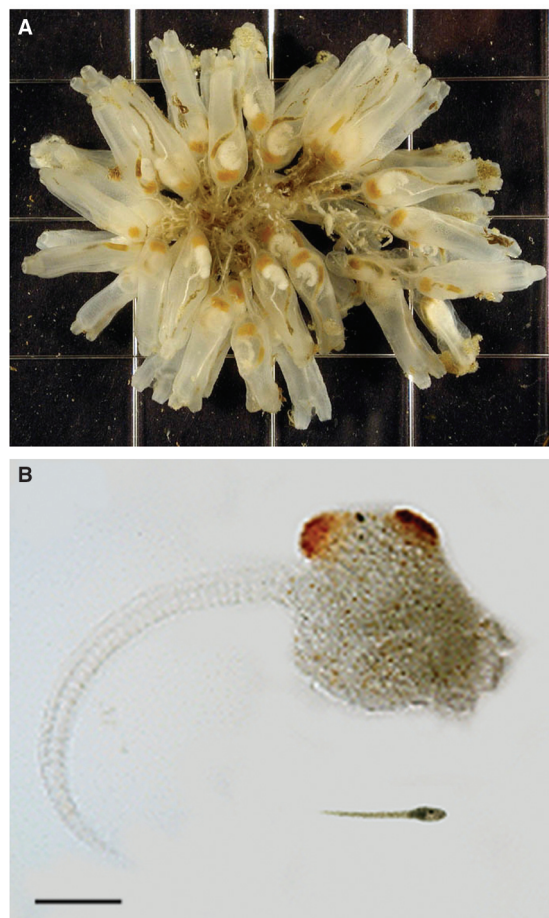
It has long been held that the neural crest is a defining feature of vertebrates. The neural crest arises at the dorsal aspect of the neural tube and then migrates widely in the embryo, giving rise to a range of derivatives which are distinctly vertebrate, such as the neurons and glia of the peripheral nervous system, melanocytes, and, additionally in the head, cartilage, bone and teeth. The other members of the phylum Chordata, the urochordates and the cephalochordates, the nearest living relatives of the vertebrates, have been thought to lack neural crest cells. Indeed, the evolution of the neural crest was believed to have been concomitant with, and pivotal to, the evolution of the vertebrates [1].

Excitingly, however, a recent paper [2] has directly challenged current received wisdom with the demonstration that urochordates possess neural crest-like cells. Thus it would seem that neural crest cells did not evolve with the vertebrates but that they have a more ancient history. The results presented here also have serious implications for how we view the relationships between the vertebrates, the cephalochordates and the urochordates, as they may suggest that it is the urochordates that are the true sister group of the vertebrates and not, as is generally accepted, the cephalochordates.

Given the importance of the neural crest to vertebrates, there have been numerous previous studies looking at how neural crest cells evolved. By and large, these studies focused on a cephalochordate, amphioxus, and on a few urochordate species, primarily *Ciona intestinalis*. They found that cells at the neural plate border in these species express orthologues of some of the genes known to be involved in specifying dorsal neural tube fates, including neural crest cells, in vertebrates [3–8]. They did not, however, find direct evidence for the existence of migratory neural crest cells. Rather, these studies suggested that the neural crest evolved, with the vertebrates, from dorsal neural tube cells, and that both are linked by a shared developmental programme.

In their search for neural crest cells in urochordates, Jeffery *et al.* [2] used the colonial ascidian *Ecteinascidia turbinata*, rather than focus on species such as *Ciona*. *Ciona* has small larvae that exhibit the conventional mode of development; the larvae exist as free swimming members of the plankton, and during metamorphosis, the larval head attaches to the substrate, the tail is lost and the tissues of the head are reorganised into a sessile filter feeder. Contrastingly, *Ecteinascidia* has a giant larva, and adult development is initiated in the head during the embryonic phase, with the pharynx, heart, siphons and body pigment cells forming precociously (Figure 1).

The fact that the *Ecteinascidia* larva is so big allowed Jeffery *et al.* [2] to look for migratory neural crest-like cells in this ascidian using a cell-tracing method used to study neural crest cells in vertebrates. They found that, if they injected the fluorescent lipophilic dye Dil into the anterior neural tube at the



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Figure 1. The urochordate *Ecteinascidia turbinata*.

(A) A colony of *Ecteinascidia turbinata*. (B) The giant tadpole of *Ecteinascidia* compared to the small tadpole of another ascidian species, *Stela clavae*.

early tailbud stage, they could, with time, observe cells migrating away from the neural primordium towards the developing siphons and body wall. Although the posterior neural tube was not found to release migratory cells at these early stages, injections at later stages did highlight the production of such cells by this region, and again these cells migrated into the body wall.

Just as in vertebrates, therefore, migratory cells emerge from the neural tube during *Ecteinascidia* development and, again like vertebrates, they do so in an anterior to posterior sequence. This cell tracing analysis also revealed a further startling feature of these migratory cells. Jeffery *et al.* [2] followed the fate of the cells that migrated to the siphons and body wall, and found that they differentiated as pigment cells. Again, this directly mirrors what is seen in vertebrates; the neural crest cells that populate the skin generate melanocytes. The authors further probed whether these migratory cells in *Ecteinascidia* expressed any of the markers associated with vertebrate neural crest cells. They found that, indeed, the pigment cells of the siphons and body wall could be highlighted using two general vertebrate neural crest markers, staining with the HNK-1 antibody [9] and expression of a *Zic* gene [10].

Collectively, these results demonstrate that ascidian urochordates possess neural crest-like cells, and they provide us with the basis of a scenario for neural crest evolution in chordates. As Jeffery *et al.* [2] suggest, the first step in the evolution of the neural crest may have been, as is seen in *Ecteinascidia*, the emergence of the capacity of the neural tube to release migratory pigment cell precursors in an ascidian-like chordate ancestor, possibly as a means of protection from the harmful effects of sunlight in a shallow marine environment. The fact that these cells are derived from the neural tube makes it fairly easy to imagine how neural crest cells could go on to form the neurons and glia of the vertebrate peripheral nervous system. The evolution of the vertebrates, however, would further require that these migratory neural-tube-derived cells further attained the ability to form skeletal tissues, and this would most likely have been driven by alterations to the embryonic environment and the corresponding response of these migratory neural derived cells.

Another very important aspect of this work is that it calls into question the relationships between the chordate subphyla: the vertebrate, the cephalochordates and the urochordates. By and large, it has been accepted that it is the cephalochordates, exemplified by amphioxus, which are the true sister group of the vertebrates (Figure 2A) [11]. The body plan of amphioxus is more vertebrate-like than that of any urochordate. If this relationship were true, then one would have to assume that neural crest-like cells evolved at the base of the chordates, and that amphioxus has secondarily lost these cells.

But it is also possible that the urochordates are the true sister group of the vertebrates, and that the cephalochordates are basal (Figure 2B). If this were true, it would suggest that neural-tube-derived migratory cells evolved in a common ancestor of the

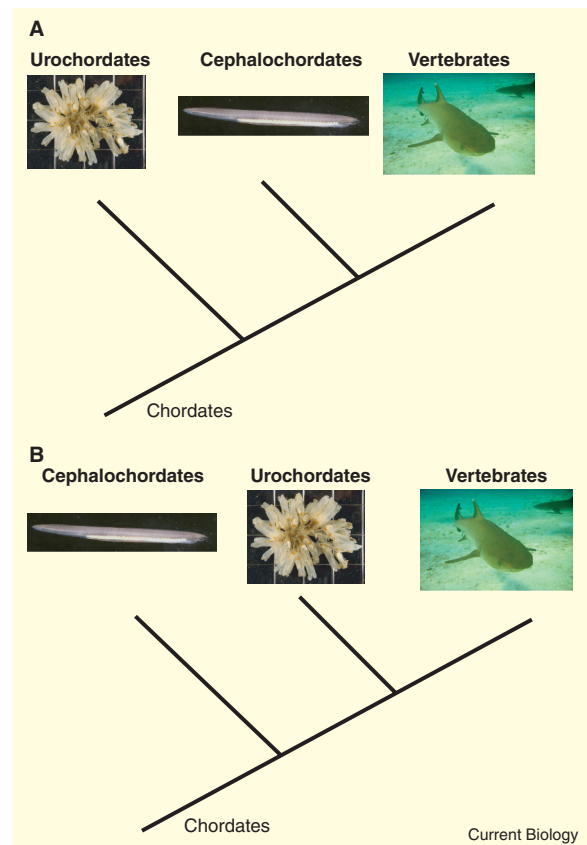


Figure 2. Chordate relationships.

(A) The accepted relationships amongst the chordates. Urochordates are basal, and cephalochordates form the sister group to the vertebrates. (B) A possible new interpretation of chordate relationships, which places cephalochordates in a basal position and the urochordates as the sister group to the vertebrates.

vertebrates and urochordates, and not at the base of the chordates. Placing the vertebrates and urochordates as sister groups may seem to fly in the face of current perceptions, but there are other lines of evidence that back this relationship. An analysis of the diversity of classical cadherins showed that cadherin genes isolated from ascidian urochordates have the same domain organisation as vertebrate cadherin, while an amphioxus cadherin has a distinct structure [12]. So it may be that long-held views on the relationships between the chordates have to be reassessed, and that the urochordates will be elevated to sisterhood with the vertebrates.

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